The Development of the Spinal Motor Column in Relation to the Myotomal Muscle Fibers in the Zebrafish (Brachydanio rerio)

I. Posthatching Development

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Summary. The neuromuscular system in the trunk of larval and adult zebrafishes was studied by means of light and electronmicroscopical methods. Spinal motoneurons were identified with the horseradish peroxidase retrograde transport method. We correlated the differentiation and growth of the myotomal muscle with the number of motoneurons per spinal cord segment and the size of the motoneuron somata.

The adult number of motoneurons is reached in an early larval stage, before the muscle fiber type differentiation in the myotomes is completed. The mean motoneuron size does not bear a clear correlation with the size of the myotomal muscle.

In adult zebrafishes we could distinguish the motoneurons which innervate the superficial slow red and the deep fast white muscle fibers on the basis of soma size and position in the motor column. The motoneurons of the red muscle part are small; they are located in the ventrolateral part of the motor column. The motoneurons of the deep fast white fibers are large; they lie near the central canal.

Key-words: Development – Spinal cord – Motoneuron – Muscle.

Introduction

The post-embryonic development of the myotomal muscle in fish is characterized by an increase in the number of histochemically defined muscle fiber types and by hyperplasia and hypertrophy of the muscle fibers (Waternman 1969; Willems and van de Berg 1978; Proctor et al. 1980; Weatherly...
et al. 1980; van Raamsdonk et al. 1978; 1982a, b, c). We are interested in the question of how the development of the spinal motoneuron population is related to these developments in the myotomes. Within this context, we concentrated on two problems:

To what extent do the spinal motoneurons determine the differentiation of the muscle fiber types?

What proportion do the number and size of the spinal motoneurons bear to the dimensions of the myotomal muscle?

Thusfar it has been assumed that the differentiation of muscle fiber types is induced by the innervating nerve (Jolesz and Srêter 1981; Salmons and Henriksson 1981; Rubinstein and Kelly 1981). Recently, the general validity of this hypothesis has been queried. It was shown that heterotopic transplantations of neural tube fragments in chicken embryos did not change the actomyosin properties of twitch and tonic muscles (Khaskiye et al. 1980). Laing and Lamb (1982) showed that transplantation of wingbuds to the lumbar area of early chicken embryos did not alter the pattern of fast and slow muscle fibers in the developing wing. We demonstrated that in fish embryos the white and embryonic red muscle fibers develop in a normal pattern, despite the removal of the spinal cord at an early developmental stage (van Raamsdonk et al. 1982b). The most plausible interpretation of these results is that, at least in lower vertebrates, intrinsic myogenic factors play a role in the differentiation of the muscle fiber types and in determining the spatial distribution of the fiber types in skeletal muscles.

If so, two questions arise. First, how are the properties of the spinal motoneurons attuned to the intrinsically determined properties of the skeletal muscle fibers, so that they develop into “red or slow” and “white or fast” motoneurons? Second, how is the number of the different types of motoneurons correlated with the number of red and white muscle fibers?

To gain insight into these problems, we investigated the neuromuscular system in the trunk of the zebrafish (*Brachydanio rerio*). For several reasons this teleost fish is an attractive object:

The architecture of the trunk musculature and the spinal cord of teleosts is relatively simple (cf. Nieuwenhuys 1964; van der Stelt 1968; Bernstein 1970; van Raamsdonk et al. 1974, 1980).

The trunk musculature of teleost fishes does not contain muscle spindles (Bone 1978), therefore motoneurons which supply the myotomal muscle will be of the $\alpha$-type.

The differentiation of the myotomal muscle fibers has been extensively studied (Waterman 1968; van Raamsdonk et al. 1974, 1978, 1982a, b, c).

The light-microscopical histology of the developing spinal cord in teleosts has been described (Gideiri 1966); unfortunately, however, little is known about the morphology of the motoneurons in developing teleost fishes.

In this morphological study we quantified the number, the soma sizes and the axon diameters of motoneurons in adult and larval zebrafishes. These data were correlated with measurements of the hyperplasia and hypertrophy of the myotomal muscle and with determinations of the muscle